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원 저

Efficacy of the Arbekacin and Teicoplanin Combination on Glycopeptide Intermediate *Staphylococcus aureus* in a Rabbit Model of Endocarditis

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토끼 심내막염 모델에서 Glycopeptide Intermediate *Staphylococcus aureus*에 대한 Arbekacin과 Teicoplanin 병용 요법의 효과

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Background : There have been no reports to evaluate the usefulness of combination therapy with glycopeptide and arbekacin in endocarditis by in vivo model.
Materials and Methods : We investigated the efficacy of the arbekacin and teicoplanin combination on glycopeptide intermediate *Staphylococcus aureus* (GISA) in rabbit model of endocardits. GISA Mu50 strain was used for the experiment. The rabbit model of aortic valve endocarditis as described previously was used. Treatment was started 20h later inoculation with teicoplanin alone (at 20 mg/kg of body weight intramuscularly every 12 hours for 4 days after loading dose of 40 mg/kg of body weight intramuscularly), arbekacin alone (5 mg/kg of body weight intramuscularly every 12h for 4 days), or teicoplanin plus arbekacin.
Results : The results of therapy for experimental endocarditis due to Mu50 showed that teicoplanin and arbekacin combination was more effective than the administration of both drugs alone in reducing the $\log_{10}$CFU/g of aortic vegetation ($P<0.05$).
Conclusion : The combination of teicoplanin and arbekacin was more effective against GISA (Mu50) than both drugs alone in vivo endocarditis model.

Key Words : Endocarditis, *Staphylococcus aureus*, Teicoplanin, Arbekacin

INTRODUCTION

The emergence of glycopeptide intermediate *Staphylococcus aureus* (GISA) has caused serious concerns about the antimicrobial treatment of *S. aureus* endocarditis. Moore et al. (1) reported vancomycin treatment failure associated with GISA in a patient with endocarditis and in the rabbit model of endocarditis. Pavie et al.(2) reported teicoplanin was approximately 100-fold less active than the standard dose of vancomycin against the GISA strain and selected for the emergence of more resistant

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subpopulations in a rabbit model of endocarditis.

The benefit of combination therapy with a cell wall active agents and aminoglycosides has not been definitively established by human clinical trials of *S. aureus* infective endocardits. However, Perry et al. (3) recommended that if a glycopeptide is indicated for the treatment of endocarditis, combination therapy with a suitable aminoglycoside should be considered.

Hanaki et al. (4) reported the synergistic interactions between arbekacin and teicoplanin against GISA strains. And, Lee et al. (5) reported that the combinations of arbekacin with vancomycin, teicoplanin, or ampicillin-sulbactam showed the synergistic interaction against hetero-GISA strains. But, no reported study has evaluated the usefulness of glycopeptide-arbekacin combination therapy in endocarditis due to GISA using an *in vivo* model.

In this study, we investigate the efficacy of teicoplanin and arbekacin on GISA in a rabbit model of endocarditis.

MATERIALS AND METHODS

1. Strains and animals

Mu50 strain (a gift from Dr. Hiramatsu) was used for the experiment. MICs were determined by broth microdilution method of the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS) (6). MIC determinations were performed using cation-adjusted Mueller-Hinton broth with an inoculums of 5×10^5 CFU/mL. Two antimicrobial agents were used in the experiment; teicoplanin (Aventis, Seoul) and arbekacin (Meiji Seika, Ltd, Tokyo, Japan). The experimental animals were 2.5 kg, white New Zealand rabbits.

2. Experimental staphylococcal endocarditis

To evaluate the efficacy of teicoplanin and arbekacin on GISA, we used a rabbit model of aortic valve endocarditis, which has been described previously (7). The animals received 50-60 mg of sodium pentobarbital intravenously and were anesthetized by ethyl ether inhalation. The right carotid artery was exposed through a 5 cm longitudinal incision in the neck, ligated and then incised. A polyethylene catheter was inserted a distance of about 9 cm until resistance was met. It was then withdrawn slightly and tied in place. The tip thus remained in a position just above the semilunar cusps of the aortic valve. The catheter was then filled with a sterile saline, and the skin incision was closed with silk over the free end of the catheter. Twenty-four hours after catheter placement, the rabbits were infected with 10^7 CFU of *S. aureus*.

3. Treatment with antibiotics and quantitative culture

The pretreatment burden of the organisms in the vegetations was determined using untreated control rabbits, which were killed 20 hours after inoculation. Treatment was started 20 hours after inoculation with teicoplanin alone (at 20 mg/kg of body weight intramuscularly every 12 hours for 4 days after loading dose of 40 mg/kg of body weight intramuscularly), arbekacin alone (at 5 mg/kg of body weight intramuscularly every 12 hours for 4 days), or teicoplanin plus arbekacin. The teicoplanin dose of 20 mg/kg every 12 hours was based on the previous report that showed a peak and trough concentration in serum of 44±5 μg/mL and 21±5 μg/mL, respectively (2). The arbekacin dose of 5 mg/kg every 12 hours was based on the previous reports that showed a 1-h postdose concentration in serum of 18 ± 0.46 μg/ml at 5 mg/kg intramuscularly (8). Treated rabbits were euthanized 20 hours after the administration of the last antibiotic dose. Aortic valve vegetations were harvested, weighed, and homogenized in 0.5 mL of 0.9% saline with a tissue homogenizer (Wheaton Science, Milleville, NJ, USA). Serial ten-fold dilutions of the homogenate were made in 0.9% saline and quantitatively cultured on Mueller-Hinton agar containing 8 μg of teicoplanin per ml. These dilutions avoided any significant in vivo carryover (9). After incubation at 37℃ for 24 hours, colonies were counted and the results obtained were expressed in $\log_{10}$CFU per gram of vegetation. The lower detection limit for this method is 1 CFU per 50 μL of undiluted vegetation homogenate.

4. Statistical analysis

Comparisons of mean bacterial counts ($\log_{10}$CFU per

Table 1. Outcome of 4-days Treatment for Experimental VISA Aortic Valve Endocarditis

	Control (N=3)	Arbekacin alone (N=3)	Teicoplanin alone (N=3)	Arbekacin plus Teicoplanin (N=3)
Mean bacterial count (log_{10} CFU/g)±SD	8.51±0.67	8.18±0.76	8.37±0.03	5.54±0.41

gram of vegetation) between groups were determined by analysis of variance (one-way ANOVA). Turkey's post hoc comparison was used. A P value≤0.05 was considered significant. Statistical analysis was performed using SPSS 11.0 (SPSS, Chicago, IL, USA).

RESULTS

The MICs of arbekacin and teicoplanin for M50 strain were 8 μg/mL and 16 μg/mL.

The results obtained using the 4-days antibiotic treatment regimen, for experimental endocarditis due to GISA M50, are presented in Table 1. A total of 12 rabbits infected with GISA M50 were assigned to the control group (n=3) and to the various treatment regimens groups (3 for teicoplain alone, 3 for arbekacin alone and 3 for arbekacin/teicoplanin). Control rabbits had a mean ±standard deviation aortic valve vegetation bacterial count of 8.51±0.67 log_{10} CFU per gram. Treatment with arbekacin alone or with teicoplanin alone did not reduce the aortic valve vegetation bacterial count significantly. However, treatment with arbekacin/teicoplanin reduced the aortic valve vegetation bacterial count significantly (P<0.05).

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major nosocomial pathogen, and community-acquired infections caused by MRSA are increasing. Glycopeptide has been the main antimicrobial agent for treating severe infections caused by gram-positive bacteria, and is used for the treatment of infections caused by antibiotics-resistant gram-positive bacteria such as MRSA, and β-lactam resistant enterococci. However, vancomycin-resistant enterococci and *S. aureus* strains with reduced susceptibilities to glycopeptides have emerged (10-12), and the emergence of GISA has raised the question of the clinical relevance of this low level of resistance and of the efficacies of glycopeptides for the treatment of infections due to GISA. However, vancomycin treatment failures and slow clinical responses in cases of *S. aureus* endocarditis have been reported (13).

Arbekacin, a derivative of the aminoglycoside dibekacin has been used in Japan to treat infections caused by MRSA. S. aureus resistant to aminoglycoside has been reported to produce the enzymes 4′-aminoglcoside adenyltransferase, 3′-aminoglycoside phosphotransferase, or 2′-aminoglycoside phosphotransferase. However, arbekacin has a low modification rate for the several aminoglycoside-modifying enzymes, so this antibiotic is expected to be used to treat MRSA infection (14, 15).

Hanaki et al. (4) reported that. there were synergistic interaction between arbekacin and teicoplanin against GISA Mu50 strain by checkerboard synergy testing with fractional inhibitory concentration (FIC) index of 0.375. Antibiotic combinations for the treatment of endocarditis should produce a rapid bactericidal effect. In experimental animals, it has been shown that the rate of bactericidal action expressed by combination of drugs in broth is predictive of the relative rate at which the organisms would be eradicated from the cardiac vegetations in vivo (16). So, the combination of glycopeptides and arbekacin could be an alternative option in the treatment of GISA endocarditis.

This study showed that the treatment with teicoplanin and arbekacin in combination may be more effective than treating with either teicoplanin or arbekacin alone in a rabbit model of endocarditis. This finding is the first report about the usefulness of glycopeptide-arbekacin combination therapy in endocarditis due to GISA using an in vivo model.

Because we used in vivo model for evaluating the usefulness of combination treatment and the definitions of synergism in this model have not been defined, we could not evaluate the synergistic or additive effects of

the combination treatment. However, we could verify that the treatment with teicoplanin and arbekacin in combination could significantly decrease the mean bacterial counts of vegetation by about 3.0 $\log_{10}$ CFU/g.

Although we did not measure the serum levels of the antimicrobial agents, the peak and trough concentrations of those agents would be higher than MICs of the agents for Mu50 strain (2, 8). However, the treatment with teicoplanin or arbekacin could not decrease the mean bacterial counts, compared with the control group. The suboptimal efficacy of those agents may be related to the maldistribution of the agents within vegetations (17). And, the bacteria in the vegetations could reach the large population.

However, there are several limitations in this study. First, the small number of animals per group were used. Although there were statistical significance between the combination therapy group and the single drug group, this is not a sample size big enough for a study from which fundamental conclusions are being drawn. Second, the serum levels of the agents were not measured and the doses of teicoplanin and arbekacin may have been lower than the therapeutic doses.

In spite of the limitations, the results of our study suggests that the combination of glycopeptide and arbekacin may be useful in the treatment of endocarditis caused by GISA. Further study should be performed about the effectiveness of glycopeptide and arbekacin combination.

요 약

목 적 : 동물 모델을 이용하여 GISA에 의한 심내막염에서 teicoplain과 arbekacin 병용 요법의 치료 효과를 알아보고자 하였다.

재료 및 방법 : 토끼 심내막염 모델을 이용하여 GISA에 의한 심내막염에서 teicoplanin과 arbekacin 병용 요법의 효과를 단독 요법의 효과와 비교하였다. GISA 균주는 Mu50 균주를 사용하였고, 기존 문헌과 동일한 방법으로 토끼를 이용하여 동맥판 심내막염 모델을 사용하였다. 항생제 치료는 균을 주사한지 20시간 이후에 시작하였다. Teicoplanin은 40 mg/kg를 1회 근육 주사한 후에 20 mg/kg의 용량을 12시간 마다 4일간 투여하였다. Arbekacin은 5 mg/kg를 12시간 마다 4일간 근육 주사하였다. 마지막 항균제 투여 20시간 이후에 토끼 대동맥판의 증식(vegetation)을 채취하였다. 항균제를 투여하지 않은 대조군, teicoplanin 단독 치료군, arbekacin 단독 치료군, teicoplanin과 arbekacin 병용 치료 군간의 증식의 무게(gram) 당 $\log_{10}$CFU의 차이를 비교하였다.

결 과 : Teicoplanin과 arbekacin 병용 치료군에서 teicoplanin 단독 치료군, 혹은 arbekacin 단독 치료군에 비해 증식의 무게(gram)당 $\log_{10}$CFU 값이 유의하게 낮았다 (P <0.05).

결 론 : 동물 모델에서 GISA에 의한 심내막염에 대한 teicoplanin과 arbekacin 병용 치료의 효과가 teicoplanin이나 arbekacin 단독 치료에 비해 우월하였다.

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